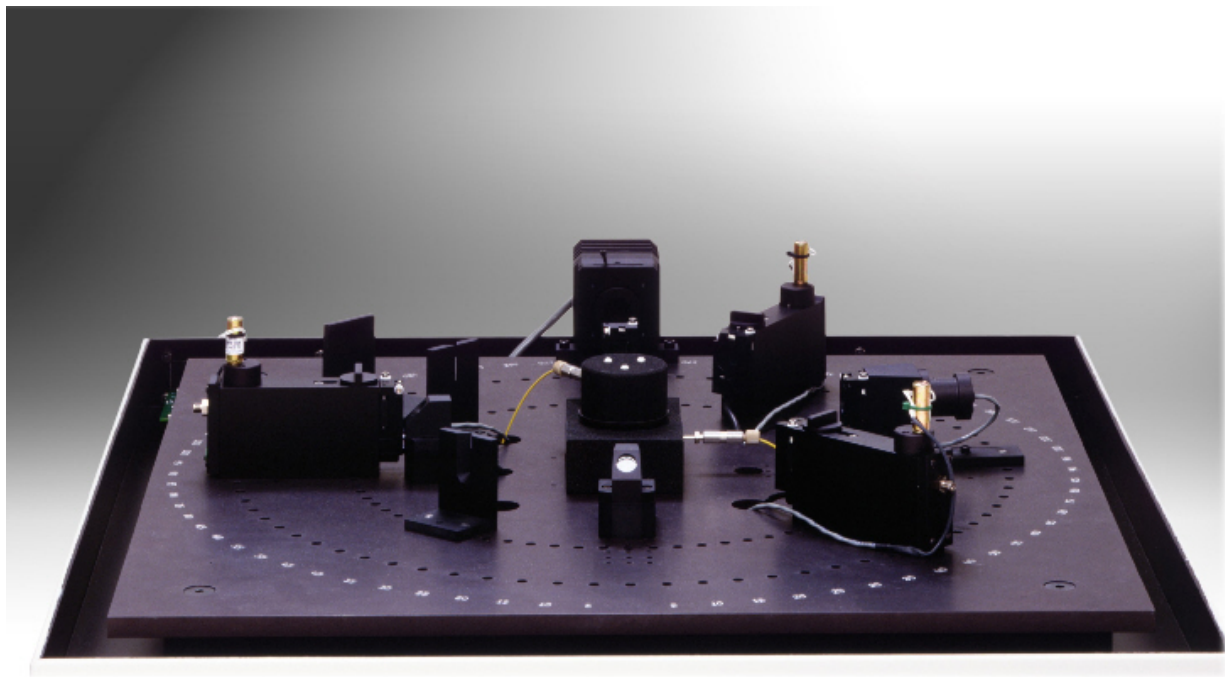


# ***PDExpert™***

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**Multi-Angle Light Scattering Workstation**

## **User Manual**



**Created by Precision Detectors, Inc.**

Notices:

This product is covered by a limited warranty. A copy of the warranty is included in this manual.

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## **Warranty**

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Precision Detectors (PDI) will repair or replace any item that does not work as specified in our User's Manual for a period of 1 year from the date of delivery of your System. This warranty does not cover damage from causes outside PDI's control if you have made any alteration to any item, if you use any item except within a liquid chromatography system and within our specified temperature. If we have delivered a computer and/or other manufacturer's liquid chromatography equipment as part of your System, you agree to accept the computer or chromatography equipment manufacturer's warranty in place of PDI's warranty.

THE WARRANTIES CONTAINED HEREIN ARE IN LIEU OF ALL OTHER WARRANTIES, EXPRESS OR IMPLIED, INCLUDING ANY WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE WHICH ARE EXPRESSLY DISCLAIMED BY PDI.

### **Repair of malfunctioning items**

In the event that your system needs repair after the 1 year warranty period, we agree to repair it for you at our then current labor and material rates if it is sent freight and insurance prepaid to our headquarters.

### **Maintenance**

For a period of 5 years following delivery of your system, we agree to clean and maintain items for you at your request charging you our then current labor and material rates if the system is sent to our headquarters, freight and insurance prepaid.

### **Limitations of liability**

PDI is not liable for any damages of any kind, incidental or consequential and under no circumstance will damages be in excess of the amount paid by you to PDI for the System regardless of the form of the claim.

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## Warnings and Safety Precautions

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The Precision Detectors PDExpert Light Scattering Workstation can be used as a detector system for High Performance Liquid Chromatography, Size Exclusion Chromatography and Gel Permeation Chromatography or it can be used as a static system with a test tube as a sample holder. The following precautions should be followed to minimize the possibility of personal injury and/or damage to property while using the instrument.

**1. Maintain a well ventilated laboratory.**

The mobile phase or solvent typically contains a volatile organic solvent. Ensure that the laboratory is well ventilated so that a buildup of vaporized solvent cannot occur.

**2. Avoid open flames and sparks.**

Do not use an open flame in the laboratory and do not install any equipment that can cause sparks in the same room as the instrument.

**3. The instrument must be plugged into a grounded power line.**

Ensure that all parts of the system are properly grounded. It is strongly recommended that all parts of the system are connected to a common ground.

**4. Treat all samples and mobile phases as if they are capable of containing hazardous substances or transmitting disease.**

The sample and/or mobile phase may contain compounds which may present a health hazard. If you are analyzing biological/clinical samples, treat them in accordance with the infectious disease control program of your institution.

**5. Do not attempt to over-ride High Voltage or Laser Safety Interlocks.**

Do not run the instrumentation unless all protective devices are functioning properly.

**6. Do not remove the cover of the system while the power is provided to the laser.**

The laser can cause serious eye damage.

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## Warning Labels

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Electrical Requirements: 100/120/230/240 V,  
50/60 Hz

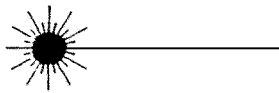
To be serviced by factory authorized personnel  
only. For assistance call (508) 966-3847 or  
your local representative



Protective conductor terminal



Earth (ground) terminal



Danger Laser radiation when open. Avoid  
direct exposure to beam.

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## Preface

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The Precision Detectors PDExpert Multi-Detector Workstation is a multi-detector system for light scattering measurements. Flowing samples (High Performance Liquid Chromatography, Size Exclusion Chromatography and Gel Permeation Chromatography) as well as stationary samples (in a test tube) can be studied. The system can provide the following types of measurements:

- Static Light Scattering to provide  $R_g$  and molecular weight measurements.
- Dynamic Light Scattering with on-board correlators and photon counting to provide flow-through  $R_h$  and Diffusion Constant measurements.

The workstation is capable of measuring the scattered light at a number of angles in a single experiment. This data can then be used for advance molecular weight measurements such as Zimm plots. A short discussion of these measurements is presented in Appendix D.

The user can configure the system to meet the exact requirements of the laboratory with powerful data acquisition, processing, reporting, storage and retrieval programs such as Precision Detectors *PrecisionMALS* and Precision Detectors *PrecisionIlluminte*. Detailed information about these programs is included in the manuals provided with those software packages.

This manual is designed to provide information about the use of the detector, sample handling, troubleshooting and related topics. It contains information about all modules that are available for the PDExpert system. For additional information about detector modules that are not installed in your detector, please contact Precision Detector or your local representative.

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# Chapter 1

## Introduction

1

### 1.1 OVERVIEW

The *Precision Detectors* PDExpert™ Light Scattering Workstation is a multi-detector light scattering platform that can be employed for light scattering measurements on a static sample (e.g. a test tube) or for flow through measurements (e.g. High Performance Liquid Chromatography, Gel Permeation Chromatography and Size Exclusion Chromatography). The workstation is designed to provide the ultimate in detection for characterization of polymers, nanoparticles, lipids, colloids and proteins.

The optical bench of the workstation is the heart of the system (Figure 1-1).

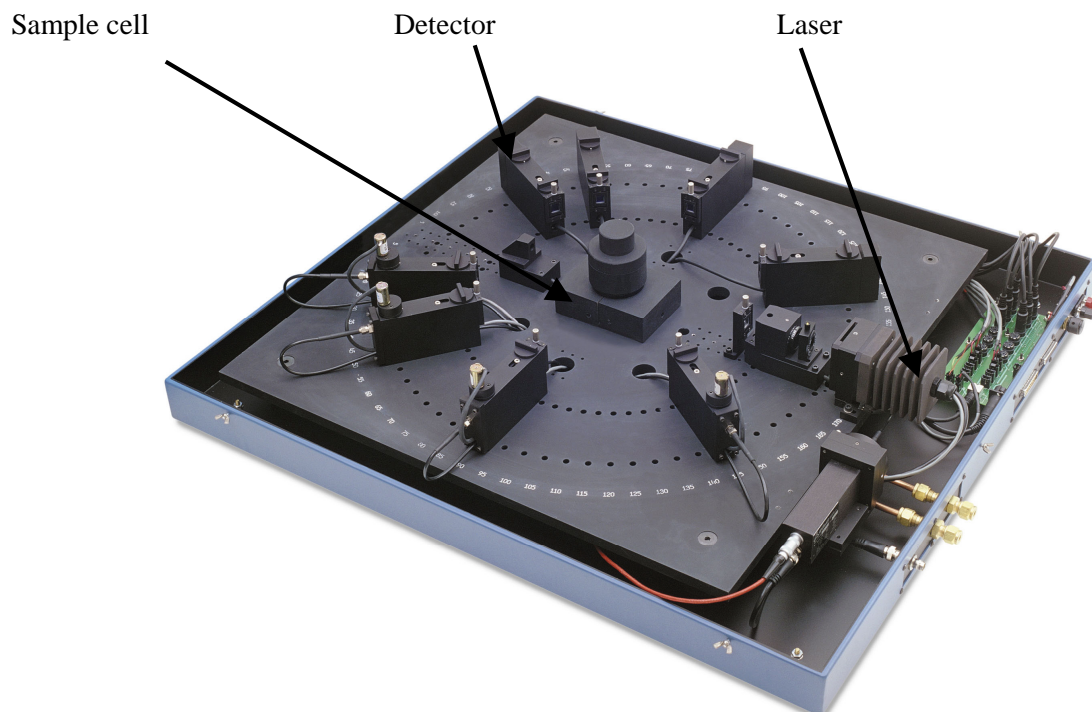


Figure 1-1: The Optical Bench of the Precision Detectors PDExpert Light Scattering Workstation

The sample holder is located in the center of the optical bench. Two sample holders are available:

- A static sample chamber (Figure 1-2), in which the sample is placed in a 6 mm disposable test tube which requires 150  $\mu\text{L}$  of sample. Sample chambers for 3 mm and 5 mm tubes as well as NMR tubes (10  $\mu\text{L}$ ) are available.

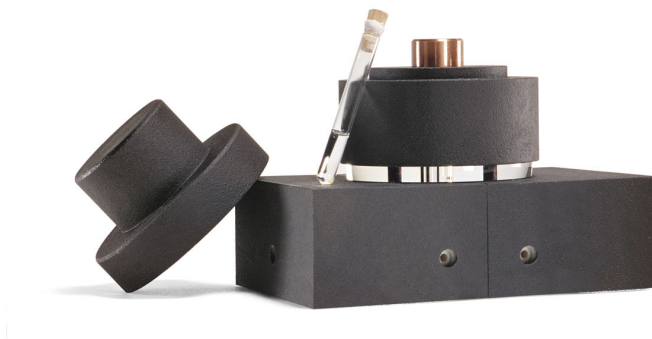


Figure 1-2: The Static Sample Chamber

- A flow cell detector which is similar to the fixed sample holder. The mobile phase is delivered through a port on the rear of the optical bench.

The base system includes a dynamic light scattering detector that is set at a user specified angle. Additional static and dynamic detectors are available from Precision Detectors (up to twenty-four static and eight dynamic detectors can be accommodated). Detectors can be set at any angle between 5 and 175° (at 5° intervals) as desired.

A typical dynamic detector module is shown in Figure 1-3; the static detector module is similar in design and is 1" longer than the dynamic detector module.



Figure 1-3: A Dynamic Detector Module Assembly

## 1.2 FEATURES OF THE DETECTOR

The PDExpert Light Scattering Workstation includes the following features:

- A diode laser (685 nm) with 30 mW power is employed. The laser is temperature controlled for stability.
- Up to 24 Static and 8 Dynamic Light Scattering angles can be selected for optimum data acquisition. The 360° base plate has three rows of threaded holes on the laser path that are 0.5" apart on 0.5" centers and 2 concentric circles of holes that are 2.5" apart drilled every 5°. These holes are precision drilled to ensure positional accuracy of the detector heads.
- A beam dump is mounted after the sample cell so that the laser beam is attenuated to essentially zero power.
- An attenuator to define the physical field of the detector and a beam monitor are available (options).
- The sample temperature can be controlled from 0-80° C via a Peltier controller.
- The flow cell module can be readily removed and a temperature controlled sample holder can be place in the system to provide a batch analysis system (full goniometer results). The batch analysis sample holder permits the use of standard test tubes for samples.
- An alignment laser (689 nm, 3 mW) is included for use in aligning the optical components to optimize performance.
- The distance of the detectors from the scattering point in the cell is 6", which provides excellent discrimination and detection characteristics at any angle on the optical table.
- Control of the system as well as data processing, storage and reporting is provided by *PrecisionDeconvolve32* (for dynamic light scattering measurements) and *Discovery32* (for static light scattering measurements).
- A broad range of accessories and custom configurations are available meet the specific needs of the laboratory. The system can be upgraded at any time.

### 1.3 STRUCTURE OF THE MANUAL

This manual contains general information about the system and detailed information about each specific detector module. The structure is as follows:

- *Unpacking and Installation (Chapter 2)* - Describes how the detector should be installed in the laboratory and provides information about initial checkout of the system.
- *General Operation of the Detector (Chapter 3)* - Presents a discussion about the use of the detector in an HPLC system. This chapter includes information about sample handling and related topics that are general in nature (rather than specific to a given module).
- *Using PDExpert (Chapter 4)* - Includes a discussion about sample handling and setting system parameters.
- *Maintenance and Troubleshooting (Chapter 4)* - Describes a series of operations that the operator should perform to optimize detector performance and determine the cause of problems.
- *Specifications (Appendix A)* - Describes the physical characteristics of the major components and provides certification information.
- *Spare Parts and Replacement Parts (Appendix B)* - Presents a list of components to maintain the system.
- *General Principles of Dynamic Light Scattering (Appendix C)* - Describes the fundamental principles of Light Scattering Measurements from a mathematical perspective.
- *Advanced Calculations (Appendix D)* - Presents a discussion of the use of multi-angle light scattering calculations to obtain additional information about the polymer.

### 1.4 FOR ADDITIONAL INFORMATION

The documentation provided with Precision Detectors *Discovery32* (static measurements) and Precision Detectors *PrecisionDeconvolve32* (dynamic measurements) application software program describe the application programs that are used to collect, process, store and report light scattering data collected with the system.

The documentation provided with Precision Detectors *PrecisionMALS* describes the application software for multi-angle light scattering analysis.



## Chapter 2

### Unpacking and Initial Installation

#### 2.1 INTRODUCTION

This chapter describes how the workstation should be set up in the laboratory and includes the following:

- Unpacking the workstation (Section 2.2)
- Power and water requirements (Section 2.3)
- Locating the workstation in the laboratory (Section 2.4).

Once the instrument is installed, alignment of various components should be performed as described in Chapter 3.

#### 2.2 UNPACKING THE WORKSTATION

Carefully unpack your shipment and inspect the contents to verify receipt of all components. The Precision Detectors PDExpert system is normally shipped in two cartons:

**Carton A** contains the Optical Bench (part number PD 2051) and the optical components that are installed on the optical bench. The lasers, the dynamic detectors, static detectors, beam dump and steering lens are mounted directly on the optical bench. The cell must be installed as described in Chapter 3.



Caution: The optical bench is heavy and special care should be taken when unpacking it.

**Carton B** contains the Electronics Module (part number PD2052) and a number of accessory items. The precise list is dependent on the configuration of the system. A typical system will include the items indicated in Table 2-1 (the user should refer to the shipping list for the precise list of components)

Table 2-1: Shipping List for PDExpert

Item	Part Number	# Supplied
Cable for PD2000/DLS to personal computer	SP2262	1
Power cords to connect modules to mains	SP2269	3
Diskette or CD with applications program		1
Flow Cell or Cuvette Holder		1
Static Detector		a
Dynamic Detector		a
Cables from Electronics Module to Light Scattering Platform		a
User Manual		1

- a) Dependent on user configuration

Carefully inspect the shipping carton and all components. If there is any damage to the carton or to any components or if any components are missing, contact both the shipping agent and Precision Detectors (or its representative) immediately to make a claim. If any parts are missing, please contact Precision Detectors customer service department (or your local representative) and indicate the missing items via the part numbers.



---

Warning: If there is any evidence that the Precision Detectors PDExpert system has been damaged in shipping, do not plug the unit into the line. Contact Precision Detectors or its representative for advice.

---

The shipping cartons should be retained as they can be used if it becomes necessary to transport the system.

## **2.3 POWER AND WATER REQUIREMENTS**

### **2.3.1 Power Requirements**



---

Warning: The Precision Detectors PDExpert System uses a three-prong power cord that includes a ground wire. The unit must be connected to a properly grounded three-prong power outlet to ensure safety and proper operation. If there is any doubt about the power supply, a qualified electrician should be contacted to ensure a properly operating and properly grounded power outlet.

---



---

Note: The PDExpert system accepts all voltages from 90 to 250 V (50/60 Hz).

---

The 120 VAC power cable consists of a 3-prong receptacle for attachment to the power inlet on the back of the optical bench and a three prong plug for connection to a standard U.S. grounded output.

The 100/230/240 VAC power cable consists of a 3-prong receptacle for attachment to the power inlet on the back of the optical bench. The other end of the cable has three color-coded wires that are used to attach to the appropriate plug. The color-coding of the wires meets ISO and VDE conventions as follows:

Earth Ground	Green with Yellow Stripe
Neutral	Blue
Line	Brown



---

Warning: The power plug should be installed by a qualified electrician and should be an approved plug (e.g., CE, TUV).

---

The power consumption of the system is approximately 5 VA.

If the workstation is used with an HPLC system, it should be connected to an electrical line that shares a common ground with other components of the chromatographic system (e.g., computers, recorders, the HPLC system controller, pump, autosampler, etc.). This will avoid "ground loops" which can create erratic results (e.g., varying background, high noise, etc.). Use a power strip to plug all HPLC components into a common ground, if necessary.

Although the workstation contains a built-in line filter to reduce interference at any input voltage, connection to an electrical line which also serves units with a large power drain or which may be subject to power surges (typical systems of this type include centrifuges, ovens, refrigerators and fume hoods) is not recommended. In addition, a surge suppressor or an uninterrupted power supply (UPS) should be used. Surge suppressors or uninterrupted power supplies designed for personal computers are suitable.

### 2.3.2 Cooling Requirements

If you intend to operate the system below ambient temperature, connect a source of cold running water to the cooling ports, either from a faucet or a closed circuit source. This running water will be used to cool the heat sink of the Peltier cooling electronics. The temperature of the water is related to the achievable temperature when you are operating below ambient temperature. In most cases, cold water from the tap is satisfactory when operating in the normal range of the instrument. If the desired temperature is not attainable using tap water, it may be necessary to chill the cooling water.

## 2.4 LOCATING THE DETECTOR IN THE LABORATORY



Note: If the PDExpert is used with an HPLC, SEC or GPC system, place the PDExpert system in a location such that the distance between the end of the column and the flow cell of the PDExpert system is minimized. This will reduce post-column band broadening effects and optimize chromatographic resolution.

The PDExpert should be placed in an area that is free from drafts or significant temperature changes. Avoid placing the system near air conditioning vents, windows, ovens, etc.

The system (and associated HPLC system, if employed) should be placed on a sturdy laboratory bench or table that provides access to all components and provides sufficient working space. The weight of the system is approximately 45 kg (100 lb) [depending on the system configuration].

## 2.5 INTERFACING THE MODULES



Caution: Do not apply electric power to the system until instructed to do so in these instructions. If power is connected while either end of the fiber optic cable is exposed to room light, high light levels may cause excessive heating and damage the detectors and/or the power supply. This damage is not covered by the warranty.



Note: It is not necessary to remove the cover of the optical bench during the installation.

The rear panel of the Electronics Module is shown in Figure 2-1, the back panel of the Optical Module is shown in Figure 2-2 and the back panel of the PD2000/DLS Module is shown in Figure 2-3.



Figure 2-1: Rear Panel of the Electronics Module

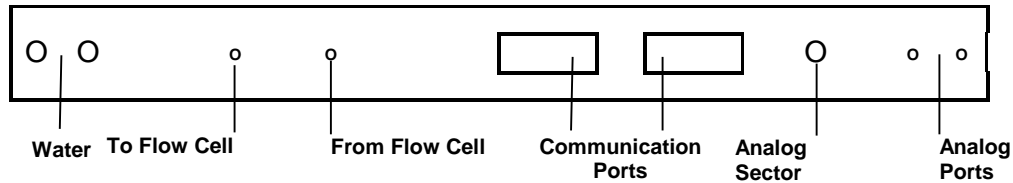


Figure 2-2: Rear Panel of the Optical Module

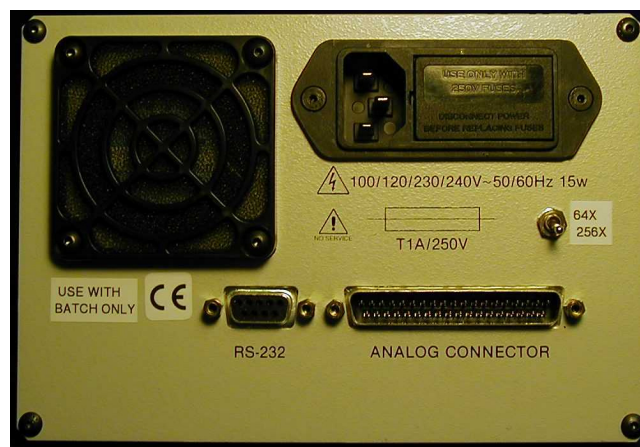


Figure 2-3: Rear Panel of the DLS/2000 Photon Detector Module

To connect the modules:

- a) Connect the fiber optic cable that is attached to the Optical Module (Figure 2-2) to the screw connector on the front of the DLS/2000 Photon Detector Module (Figure 2-3). Screw the cable onto the connector by applying it to the connector and turn it back one turn until it mates with the thread and screw it in finger tight.



Caution: Do not over tighten this connector, a finger tight connection is sufficient. Do not use a tool to tighten this connector.

- b) Check that the Batch Mode/Flow Switch on the rear panel of the DLS/2000 Photon Detector Module (Figure 2-3) is set to the 256x position. This configures the correlator to have 256 real channels in the 1024 channel space.

- c) Connect the USB cable from the Photon Detector Module (Figure 2-3) to a USB socket on the personal computer.
- d) Connect the communication cables between the Electronic Module and the Light Scattering Module. There are two cables which are keyed to fit in the appropriate manner.
- e) If you anticipate the use of the Peltier system to cool the sample cell, connect a source of cold water to the inlet on the optical bench and connect the outlet to the waste line or the re-circulating bath.
- f) If the HPLC flow cell is employed, connect the end of the column to the inlet on the optical bench and connect the outlet to a suitable waste bottle. It is recommended that the distance between the end of the column and the inlet of the cell is minimized to reduce post column band broadening.



---

Note: When you are fitting the inlet from the HPLC and the outlet line, tighten the fittings finger tight and check that they do not leak. If leaks are observed, tighten the offending fitting approximately 1/8 of a turn.

---



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Note: Do not over-tighten the fittings. If the fitting is over-tightened, it is possible that you will permanently distort the fitting, rendering it useless.

---

Once the fittings are properly made, allow mobile phase to flow through the system for 15-30 minutes at a flow rate that is 25 % greater than the flow rate that you expect to use for normal operation to ensure that there are no leaks.

- g) Connect the power cords and power up each module. The green light on each module will be illuminated.

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## Chapter 3

### Aligning the System

---

#### 3.1 OVERVIEW

This section document describes the alignment of various components of the Precision Detectors PDExpert system that are attached to the optical bench, including the laser, the cell, the steering lens and the detectors. The alignment should be performed by an individual who has been trained in the procedure. Once a system is installed, these various adjustments need not be performed unless a component is replaced or if significant deterioration of the performance has been observed.



---

Caution: The procedures in this section are performed with the power on. Take care that you do not contact live components in the controller or in the optical module.

---



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Caution: The main laser and/or the alignment laser must be powered up for certain of the procedures described in this section. When a laser is on, wear protective eyeglasses and do not look directly at the laser beam.

---

The alignment of the system includes the following steps

- *Laser Power* Calibration (Section 3.2)
- *Main Laser* Alignment (Section 3.3)
- *Alignment Laser* Alignment (Section 3.4)
- *Initial Cell* Alignment (Section 3.5)
- *Temperature* Calibration (Section 3.6)
- *Main Beam Dump* Alignment (Section 3.7)
- *Alignment Beam Dump* Alignment (Section 3.8)
- *DLS Detector* Focus and Alignment (Section 3.9)
- *Static Detector* Focus and Alignment (Section 3.9)
- *Cuvette* Alignment (Section 3.10)
- *Installing/Replacing Apertures* (Section 3.11)

When the system is initially installed, these procedures should be performed in the indicated order. These procedures can also be used in conjunction with the maintenance and troubleshooting procedures described in Chapter 4.

If a complete system alignment is performed, remove all detectors and beam dump monitors from the optical table. The various sections will indicate the components are to be installed. If these procedures are employed to check and realign a single component, remove only those components indicated in the appropriate section.

### 3.2 LASER POWER CALIBRATION

The following equipment is required to calibrate the laser power:

- Laser Power Meter
- Multi-meter
- Allen Wrench Set

To calibrate the laser power:

- a) Remove the cover of the PD4001 Electronics Module.
- b) Check that the jumper on J14 connects pins 2 and 3 (Figure 3-1).

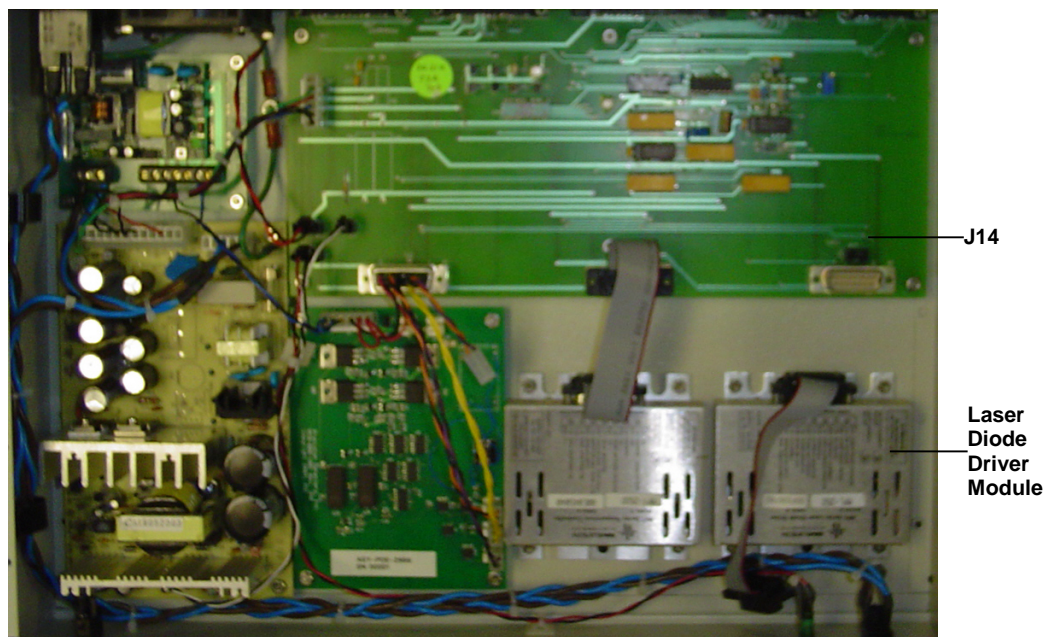


Figure 3-1: Electronics Module Printed Circuit Board



- c) Use the application software to set the *Laser Scale* to 0 and the *Laser Threshold* to 0.
- d) Turn the *Output Current Adjust* potentiometer on the laser driver board to the maximum clockwise position (all the way up).
- e) Turn the *Limit Current Adjust* potentiometer on the laser driver board to the maximum counterclockwise position (all the way down).
- f) Override the laser interlocks.
- g) Power up the laser (the laser will not appear to be on).




---

Caution: When the laser is on, wear protective eyeglasses and do not look directly at the laser beam.

---

3

- h) Turn the *Limit Current Adjust* potentiometer until a power reading of 0.69 mW is obtained. The power reading is obtained by monitoring the voltage between TP1 and GND (J13, pin1).
- i) Turn the *Output Current Adjust* potentiometer to the maximum counterclockwise position (all the way down).
- j) Turn the *Output Current Adjust* potentiometer until a power reading of 0.1 mW is obtained.
- k) Measure and record the voltage between TP1 and GND (J13, pin 1). Record this as the 0 % Voltage.
- l) Adjust the *Output Current Adjust* potentiometer until a power reading of 30 mW is obtained. The power reading is obtained with the laser power meter.
- m) Measure and record the voltage between TP1 and GND (J13, pin 1). Record this as the 100% voltage.
- n) Turn the *Output Current Adjust* potentiometer to the maximum counterclockwise position (all the way down).
- o) In the software, set the *Laser Threshold* to the voltage that you obtained in step (k).
- p) In the software, set the *Laser Scale* to the difference between the 100% voltage (step m) and the 0% voltage (step k).
- q) In the software, set the *Laser Power* to 0%.
- r) Measure the voltage between TP1 and GND. This voltage should equal the 0% voltage that was found in step k. If this voltage does not correspond to the voltage found in step k, adjust the *Laser Threshold* value in the software until the voltage between TP1 and GND equals the 0% voltage.
- s) In the software, set the *Laser Power* to 100%.
- t) Measure the laser power with the Laser Power Meter. The reading should be 30 mW. If it is not, adjust the *Laser Scale* in the software until it does.
- u) Use the application software to set the *Laser Power* to 50%.

- v) Measure the laser power with the laser power meter. The reading should be 15 mW. If it is not, adjust the *Laser Scale* to obtain the desired reading (in most cases, it will be necessary to decrease the *Laser Scale*).



---

Note: When you change the *Laser Scale*, it is necessary to change the *Laser Threshold* by the same amount. As an example; if the *Laser Power* is found to be 12 mW (instead of 15 mW) when the *Laser Scale* is 0.677 and the *Laser Threshold* is 0.378, it will be necessary to decrease the *Laser Scale*. In this case, you might lower the *Laser Scale* to 0.650; and add the value that you subtracted (0.027) from the *Laser Scale* to the *Laser Threshold*, which would now become 0.405.

---

- w) Repeat step v until the observed *Laser Power* is 15 mW.
- x) In the software, set the *Laser Power* to 25% and measure the laser power with the laser power meter. The observed reading should be 7.5 mW; if it is not, adjust the *Laser Scale* to obtain the desired reading (in most cases, it will be necessary to decrease the *Laser Scale*). When you change the *Laser Scale*, recall that it is necessary to change the *Laser Threshold* by the same amount as described above.
- y) Check the laser power at 10%, 25%, 50%, 75% and 100%. If the values are not correct, adjust the *Laser Scale* and *Laser Threshold* until the readings are correct.

### 3.3 MAIN LASER ALIGNMENT

To align the main laser:

- a) Remove the Cell Assembly, the Beam Dump Monitors and the Steering Lens from the optical platform.
- b) Loosen the screws that attach the laser to the optical platform and move the laser so that is at the maximum position from the center of the plate. Retighten the screws.
- c) Place the Alignment Target Assembly at the 0° position (directly across from the laser).
- d) Turn the laser on and set it to 10% using the application software.
- e) Override the laser interlocks.

3



---

Caution: When the laser is on, wear protective eyeglasses and do not look directly at the laser beam

---

- f) Verify that the beam is:
  - Collimated - the diameter of the beam should be the same at the laser and the target.
  - Level - the height of the beam should be the same height from optical platform at the laser and the target. A white business card is useful for this test. The card should be placed about 10 cm from the laser and about 10 cm from the target.
  - Straight - the beam should be centered on the target.

If the laser is perfectly collimated, ignore step g and continue with step h.

g) If the laser is not perfectly collimated:

- 1) Loosen the two set screws on the side of the laser and the four screws on the front face of the laser (Figures 3-3).

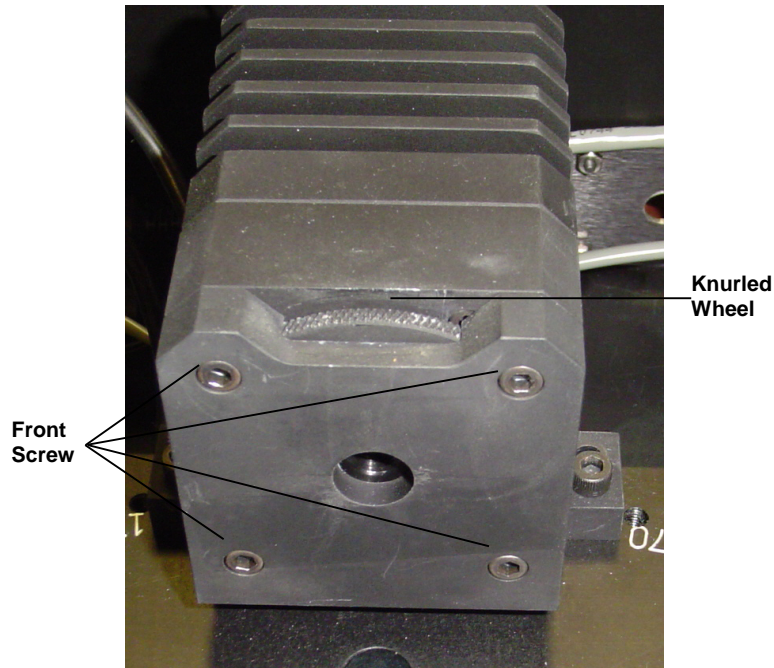


Figure 3-3: Front of Laser Housing

- 2) Turn the knurled wheel on the laser to adjust the focus.
- 3) Tighten the side set screws and the screws on the front and check the collimation.



Note: This adjustment may require a number of iterations may be a trial and error exercise, as tightening the screws will make small changes in the focus. It may be necessary to position the beam slightly off-focus when you start to tighten the screws so that the focus will be correct when the screws are tightened.

- h) When the beam is collimated, level and straight, turn the laser off.
- i) Place the steering lens on the optical platform on the set of holes nearest the center of the platform on the same side as the laser is positioned. The curved surface of the lens should be facing the laser.
- j) Turn the laser on and view the focus. The laser should be focused at the center of the optical platform. Move the steering lens toward the laser until you find the set of holes that provides the best focus.
- k) Turn the laser off.
- l) Secure the steering lens to the optical platform.
- m) Turn the laser back on. Although the beam is no longer level, it still needs to be straight (centered on the center of the target). Use the screw on the side of the steering lens (Figure 3-4) to adjust the beam so it is centered on the target.

3

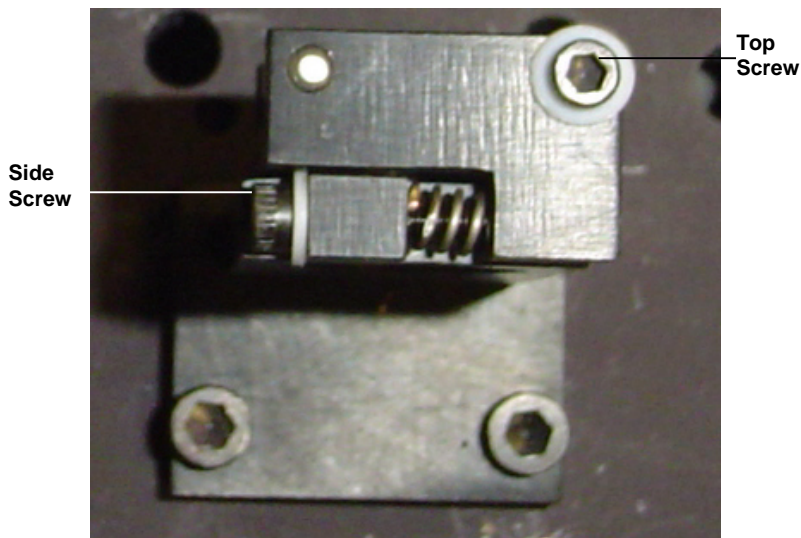


Figure 3-4: Steering Lens Adjustment Screws

### 3.4 ALIGNING THE ALIGNMENT LASER

To align the Alignment Laser:

- a) Remove the Cell Assembly and the Alignment Beam Dump Monitor from the Optical Platform.
- b) Remove the front lens from the alignment laser.
- c) Place the alignment laser at the 95° position.
- d) Place the Alignment Slot at 85° (across from the Alignment Laser)
- e) Turn the Alignment Laser on.



---

Caution: When the laser is on, wear protective eyeglasses and do not look directly at the laser beam

---

- f) Verify that the beam is elliptical with the long side of the ellipse parallel to the plate. If the beam needs to be rotated, loosen the set screw on the top of the alignment laser and twist the laser cable slightly to rotate the beam.
- g) Verify that the beam is level (it should be the same height at the laser and at the target slot) and straight (centered on the slot). If it is not, continue to move the laser with the cable until it is level, then tighten the set screw.
- h) The beam should be focused in the center of the plate (center of the copper piece) when properly focused with the lens installed. To focus the beam:
  - 1) Turn the laser off and place the lens in front of the laser (as if it were being reattached, but do not screw it in).
  - 2) Turn the laser on and check the focus. If it focuses too soon (before the plate), adjust the focusing screw counterclockwise; if it focuses too late (past the plate), adjust the focusing screw clockwise.
  - 3) To adjust the focusing screw, turn the laser off and move the lens away from the laser assembly. Inside the laser assembly, there is a ring with 2 small holes in it (this is the focusing screw).
  - 4) Use a pick or very small screw driver to turn the screw in the proper direction. Put the lens back in front of the laser and turn the laser on again.
  - 5) Recheck the focus. Continue doing this until the focus is correct. It may be necessary to perform this operation several times to optimize the beam focus.
- i) Reattach the focus/steering lens. The beam should still be centered on the target slot. If it is not, use the screw on the side of the lens to center the beam.

### 3.5 INITIAL CELL ALIGNMENT

To perform the initial cell alignment:

- a) Turn off the main laser and the alignment laser.
- b) Place the alignment target at 0° and the Alignment Slot at 85°.
- c) Put a thin layer of thermal grease on the copper plate in the center of the optical bench (this is the heat sink). The layer should be just enough to coat the plate; you should still see the copper color of the plate.
- d) Put a thin layer of thermal grease on the bottom of the cell.
- e) Place the cell on the copper plate so that the thermistor wires are toward the main laser.
- f) Attach the cell to the copper plate with 4 screws, but do not tighten them.
- g) Fill the cell with Toluene. If the cell holder is for a flow cell, use a syringe or pump to fill the cell and make sure there are no air bubbles. If the cell holder is for a cuvette, fill the cell with toluene using a pipette until the meniscus is just above the top of the quartz ring.



---

Caution: Toluene is flammable and is toxic. Wear gloves when handling the liquid and avoid getting it on your skin.

---

- h) Turn both lasers on and set the main laser power to 10%.



---

Caution: When the laser is on, wear protective eyeglasses and do not look directly at the laser beam

---

- i) Check that the main laser beam is centered on the target.
- j) Check that the alignment laser is aligned on the target. If either laser beam is not centered, slide the cell slightly on the copper plate until they are both centered.
- k) Tighten the 4 screws.



---

Note: The beam may be a line with a bright center. This is OK.

---

### 3.6 MAIN BEAM DUMP MONITOR ALIGNMENT



Note: The cell must be properly aligned (Section 3.5) before this operation is performed and the cell must be full of toluene

To align the main beam dump monitor:

- a) Make sure the laser is off.
- b) Set the switch on the front of the PD4001 to *Coarse*.
- c) Place the Beam Dump Monitor over the set of holes closest to the cell (do not secure with screws).
- d) Turn on the Main Laser and set the power to 10%.



Caution: When the laser is on, wear protective eyeglasses and do not look directly at the laser beam

- e) Move the beam dump monitor away from the cell until you find the set of holes that gives you a solid red light on the LEDs that are perpendicular to the main laser beam (D36) on the circuit board next to the optical platform (Figure 3-5).

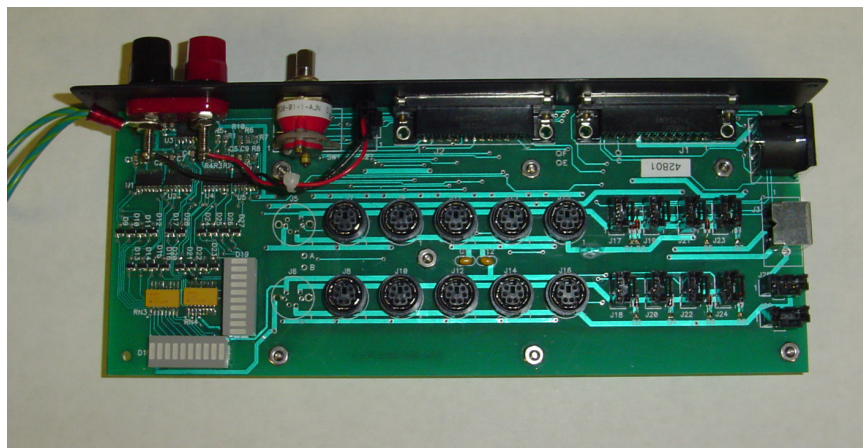


Figure 3-5: Control Circuit Board

- f) Secure the beam dump monitor.
- g) Loosen the 2 screws on the top of the beam dump monitor.
- h) Set the switch on the front of the PD4001 Electronics Module to *Fine*.
- i) Slide and twist the top of the beam dump monitor until the center LED is green.
- j) While holding the top of the beam dump monitor in place, set the switch to *Coarse* and make sure that the LED is still red. If it is no longer red, try to get it back to red by moving the top of the monitor.
- k) Set the switch back to fine. Once you get the LEDs to be Green in the *Fine* and Red in *Coarse* settings, secure the top of the beam dump monitor.





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Caution The beam dump monitor should not move when you secure the screws. Make sure that it is still Green in Fine and Red in Course once it is secured.

---

### **3.7 ALIGNMENT BEAM DUMP MONITOR ALIGNMENT**



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Note: The Cell Alignment must be completed first and the cell must be full of toluene

---

To Align the Alignment Beam Dump Monitor:

- a) Make sure the alignment laser is off.
- b) Set the switch on the front of the PD4001 to Coarse.
- c) Place the Beam Dump Monitor over the set of holes across from the alignment laser. Secure the Beam Dump Monitor to the platform.
- d) Turn on the Alignment laser



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Caution: When the laser is on, wear protective eyeglasses and do not look directly at the laser beam

---

- e) Monitor the set of LEDs that are perpendicular to the alignment laser beam (D35) on the circuit board next to the optical platform, while adjusting the height of the beam using the screw on the top of the alignment laser lens until you get a Red LED.
- f) Loosen the 2 screws on the top of the beam dump monitor.
- g) Set the switch on the front of the PD4001 box to Fine.
- h) Slide and twist the top of the beam dump monitor until you get the center LED to be Green.
- i) While holding the top of the beam dump monitor in place, set the switch to coarse and see that it is still red. If the led is no longer red, move the top of the monitor and set it back to fine.
- j) Once you get the LEDs to be Green in Fine and Red in Course, secure the top of the beam dump monitor.



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Caution The beam dump monitor should not move when you secure the screws.

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Be aware that it cannot move when you secure the screws. Make sure that it is still Green in with the Fine setting and Red in Coarse setting once it is secured.

### 3.8 DLS DETECTOR FOCUS AND ALIGNMENT

If a flow cell is employed, the cell should be full of toluene

If a cuvette is employed, it should be filled with a strong scattering sample (ex. 5mg/mL BSA) and should be centered in the cell holder

To focus and align a DLS Detector

- a) Place the detector at the angle you want and secure the detector to the optical platform.
- b) Remove the rear aperture holder and insert the eye-piece.
- c) Turn the main laser and set the power to 10%.



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Caution: When the laser is on, wear protective eyeglasses and do not look directly at the laser beam

---

- d) Hold a piece of white paper over the eye-piece and make sure you do not see a red laser spot. If you see a red spot, determine the cause and rectify the condition before continuing.
- e) Look through the eye-piece. You should see the dark edges of the cuvette and the beam across the middle. Use the screw on the top of the detector body to adjust the focus. The beam should be a then bright line and you will see some dust spots when properly focused. Secure the focus screw.
- f) Look through the eye-piece. Use the screw on the side of the detector lens to center the vertical eye-piece cross-hair between the 2 sides of the cuvette.
- g) Use the screw on the top of the detector lens to line up the horizontal eye-piece cross-hair with the laser beam.
- h) Remove the eyepiece and replace the aperture holder.

### 3.9 STATIC DETECTOR ALIGNMENT

If a flow cell is employed, the cell should be full of toluene

If a cuvette is employed, it should be filled with a strong scattering sample (ex. 5mg/mL BSA) and should be centered.

To align a static detector:

- a) Place the detector at the desired angle and secure to the optical platform.
- b) Remove the rear aperture holder and insert the eye-piece.
- c) Power up the main laser and set the power to 10%.
- d) Hold a piece of white paper over the eye-piece and make sure you do not see a red laser spot. If you see a red spot, determine the cause and rectify the condition before continuing.
- e) Look through the eye-piece. You should see the dark edges of the cuvette and the beam across the middle. Use the screw on the top of the detector body to adjust the focus. The beam should be a then bright line and you will see some dust spots when properly focused. Secure the focus screw.
- f) Look through the eye-piece. Use the screw on the side of the detector lens to center the vertical eye-piece cross-hair between the 2 sides of the cuvette.
- g) Use the screw on the top of the detector lens to line up the horizontal eye-piece cross-hair with the laser beam.
- h) Remove the eye-piece and replace the aperture holder.

### 3.10 CUVETTE ALIGNMENT



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Note: The initial Cell Alignment (Section 3.5) must be completed first

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To align the cuvette:

- a) Fill the cell with Toluene



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Caution: Toluene is flammable and is toxic. Wear gloves when handling the liquid and avoid getting it on your skin.

---

- b) Insert the cuvette so that it is just off the bottom of the cell and secure with the set screw.
- c) Turn on the Main Laser and set the power to 10%
- d) Turn on the Alignment Laser
- e) Set the Switch to Fine
- f) Use the micrometers to move the cuvette until both LED banks are in the Green (Figure 3-9)

# Chapter 4

## Using PDExpert

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### 4.1 OVERVIEW

PDExpert is a multi-detector system which can be used for measuring light scattering from a static sample (using a test tube) or a flowing sample (High Performance Liquid Chromatography (HPLC), Size Exclusion Chromatography (SEC), and Gel Permeation Chromatography (GPC)). This chapter presents information about using the system to collect light scattering data and focuses on sample handling and general operation of the system.

For additional information the user is referred to the manuals describing the application software.

### 4.2 POWERING UP THE SYSTEM

To power up the system, turn on the switches on the PD4001 Electronics Module, the PD2000/DLS Module and the personal computer. Open the application software and select the appropriate settings for the desired analysis.



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Note: For optimum results, a warm up period of 20-30 minutes is recommended.

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### 4.3 SAMPLE HANDLING

#### 4.3.1 Using the Flow Cell

When the flow cell is installed, the sample is delivered by an HPLC (SEC, GPC) system via the inlet fitting on the rear panel of the optical module. The following precautions should be followed:

- The mobile phase should be filtered through a 0.22  $\mu\text{m}$  Nylon or PVDF membrane. Make certain that the filter is compatible with all constituents of the sample.
- The sample to be analyzed should be dissolved in a suitable solvent and must be soluble in the mobile phase used for the separation. If a gradient is employed, the sample should be soluble in the entire range of the gradient that is employed. It is recommended that you check the solubility of the sample in each mobile phase using a beaker, rather than via the chromatographic system.



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Note: If a gradient mobile phase is employed, make certain that any buffers, salts, etc are soluble in all combinations of the mobile phase. We recommend that you check the solubility using a beaker or test tube, rather than via the detector, as precipitation of the buffer or salt inside the detector cell may necessitate a tedious cleaning process or a service call.

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- High purity solvents (HPLC grade) should be used when possible to prepare samples and for the mobile phase.
- The mobile phase should be degassed so that air bubbles cannot form in the flow cell.

#### 4.3.2 Using the Test Tube Chamber

When the test tube chamber is installed, the sample (150  $\mu$ L minimum) is placed in a standard disposable 6 mm test tube and the tube is placed in the sample chamber. The test tube holder is accessed by opening the hinged lid of the optical bench.



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Caution: The laser can cause serious eye damage. Always wear safety glasses when opening the cover

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Note: Ensure that the lid is closed before collecting measurements.

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Note: It is critical that the sample and the test tube are clean and there is no particulate matter in the sample. Dust, undissolved sample and any extraneous matter will significantly affect the accuracy of your results.

---

The following actions will assist you in obtaining the best results:

- Make sure the sample is well dispersed.
- Filter out any dust particles and un-dissolved materials using a filter (size?) that will not remove your sample or dissolve in the solvent. If you cannot use a filter because your sample adheres to it, filter the solvent before preparing the solution. As an alternative, centrifuge the sample to remove particulate matter (15 min at 5000g should be sufficient).
- High purity solvents (HPLC grade) should be used when possible to prepare samples, wash test tubes, etc.
- Remove any air bubbles in the sample before measurement. Air bubbles may form if the sample has been sitting for an extended period of time.
- If sample gets on the outside of the test tube, rinse with clean solvent and dry with a lint-free tissue. Do not “air dry” the tube unless you are certain that the solvent is clean.
- Do not get fingerprints in the measuring area of the test tube (lower 6 cm [1.5”]). If you have touched the test tube in the measuring area, wipe the tube with a methanol soaked lint free tissue. Fingerprints will scatter light and create scatter signals that are not related to your sample.

- Do not use scratched test tubes as the scratches can scatter light.
- Keep the sample covered at all times.
- Pour the sample into the test tube and place the test tube into the sample chamber (do not pour the sample into the test tube when it is in the sample chamber as a spill could contaminate the sample chamber). Use a narrow tipped syringe to deposit the sample at the bottom of the test tube.
- Take every other precaution to ensure that your sample is clean and free of particulate matter.

A variety of sample chambers are available including ones for 3 and 5 mm NMR tubes, which require a 10  $\mu$ L sample. Please contact Precision Detectors for additional information.

#### 4.4 SETTING OPERATING CONDITIONS

A detailed discussion about operating conditions is presented in the manuals supplied with the application software packages. The *PDExpert* dialog box (Figure 4-1) is used to select the temperature of the cell.

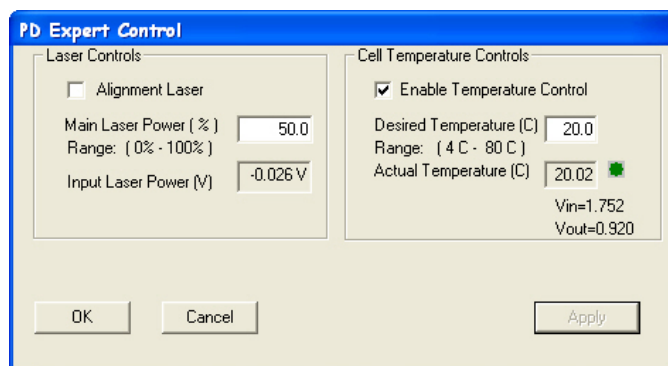


Figure 4-1: PD Expert Control Dialog Box

The *Cell Temperature Controls* field is used to control the temperature of the cell. Enter the desired temperature and press **Apply**. The temperature will be sent to the indicated temperature and the present temperature will be indicated in the Actual Temperature field. The green indicator field will be illuminated from time to time to denote that the temperature is being updated.

The *Laser Controls* field is used to set the relative intensity of full power that the laser is generating (it does not imply full power of a fraction thereof). This value will be adjusted downwards if too many photons are being received (overload). Overload may occur when the sample particles are large; the sample concentration is high and/or when low angle scattering is used. This is to be performed by an authorized service representative.



Caution: Use caution when aligning the laser to prevent exposure of your eyes to the laser beam.



Note: When a non-ambient temperature is employed, make certain that sufficient time is provided to ensure that the sample has attained the desired temperature. This issue is especially important when the sample chamber is used with test tubes.

## **4.5 COLLECTING LIGHT SCATTERING DATA**

At a given time, one dynamic light scattering measurement and up to eight static measurements can be performed. Dynamic light scattering data is initially processed via the PD2000/DLS Module and static light scattering data is initially processed by the work station.

If desired, you can independently monitor one channel of static light scattering data using the contacts on the right side of the electronics module. The switch immediately to the left of the contacts is used to select the static detector to be monitored.

From time to time, standards should be run to determine if the system is operating in an acceptable manner. For dynamic measurements, see Section 5.6, for static measurements, see Section 4.6.2.

If the signal for a given sample has decreased, it may be necessary to realign the corresponding detector module; if the signal for all samples has decreased, it may be necessary to realign the laser Contact Precision Detectors or your local representative for assistance. .

The position of the detector modules is specified by the user and set at the time of manufacture. If desired, you can move detector modules as described in Section 5.3.3 and 5.3.4.

The Expert workstation is capable of collecting light scattering data at a number of angles simultaneously. A discussion of the use of the system is presented in the software manual and a description of how the multi angle data can be employed is presented in Appendix D.



# Chapter 5

## Maintenance and Troubleshooting

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### 5.1 INTRODUCTION

Optimum performance of the Precision Detectors PDExpert detector system will be obtained when the user performs a series of routine maintenance activities on a periodic basis. This chapter provides:

- A listing of various activities that should be performed on a routine, scheduled basis (Section 5.2).
- A discussion about troubleshooting (Section 5.3).

When the system is initially installed, the analyst should obtain data (chromatogram) from a well-defined sample before developing a new analytical procedure. This data (chromatogram) can serve as a benchmark to be used to check the performance of the system. Similarly, if problems are observed in the use of a specific analytical procedure, it may be useful to use the standard sample to ensure that the system is functioning properly.

The user is encouraged to maintain a log of all operations of the detector, maintenance activities and all observed problems should be entered into the log. A discussion of the log is provided in Section 5.2.8.



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Note: The information in this chapter is designed to provide general information about the detector. Specific information about troubleshooting/maintenance for a specific mode of operation is provided in the chapter that describes the detection mode.

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### 5.2 MAINTENANCE

#### 5.2.1 Overview

While the Precision Detectors PDExpert system requires little day-to-day maintenance, we recommend that:

- Samples should be free of particulate matter (filtering through a 0.22  $\mu\text{m}$  Nylon or PVDF membrane filter is a useful method). Filters should be checked to ensure that extractable materials are not present and they are compatible with all constituents of the sample.
- The mobile phase should be filtered through a 0.22  $\mu\text{m}$  Nylon or PVDF membrane. Make certain that the filter is compatible with all constituents of the sample.
- If the Precision Detectors PDExpert system is used as a part of a chromatographic system and the output from the detector reflects the performance of the overall system, it is important to perform all maintenance procedures for each of the various components (e.g. the solvent delivery module, injector, etc.) on a routine basis. The user should refer to the operating manuals for each part of the system and perform the necessary activities on a periodic basis.

## 5.2.2 Maintenance - For Systems with a Flow Cell



Note: The frequency for doing the various activities is dependent on the sample type, mobile phase composition, sample cleanliness and a number of other factors. The frequency indicated below should be considered as a guideline. As the user gains experience with the system and the analytical procedure, it is likely that a user-generated protocol will be developed.

### 5.2.2.1 Daily Activities

For systems with a flow cell (or every time that the unit is started up):

- a) Check that the pump is working properly and the solvent bottle(s) contain sufficient mobile phase for the expected analysis.
- b) There is sufficient pump seal wash solution (if applicable).
- c) The pump seal wash system is primed and flowing properly (if applicable).
- d) All connections are leak free. Check for the presence of salt on joints and the base of all components. If a salt deposit or leak is observed tighten the offending joint (but do not overtighten; if necessary make a new fitting).
- e) If an autosampler is in the system, check that the tray temperature is correctly set, the syringe is bubble free and the wash syringe has sufficient wash solution for the day's analyses.
- f) The filters in the solvent bottle and the mobile phase should not include any particulate matter. Replace solvent filters if they are discolored (the mobile phase should be filtered with a 0.2 micron filter membrane).
- g) Run a test run using a standard or a well-defined sample and ensure that the signal has not changed appreciably from day to day.
- h) Monitor the pressure and ensure that it has not changed significantly from the level that was observed on the previous day.

### 5.2.2.2 Weekly Maintenance

For systems with a flow cell, the following should be performed on a weekly basis:

- a) Check (and replace, if necessary) any filter elements in the HPLC system.
- b) Replace the pump washing solution (if applicable).
- c) Perform a flow rate check on the pump.
- d) Perform all of the daily activities

#### **5.2.2.3 Monthly Maintenance**

For systems with a flow cell the following should be performed on a monthly basis:

- a) Inspect the condition of the tubing to detect potential problems and replace if necessary.
- b) Perform all of the daily and weekly activities.

#### **5.2.2.4 Quarterly Maintenance**

For systems with a flow cell the following should be performed on a quarterly basis:

- a) Inspect and change the seals, check valves and pistons in the solvent delivery system (if necessary).
- b) Replace the 10  $\mu\text{m}$  mobile phase filters.
- c) Replace all filter elements in the HPLC system.
- d) Perform all of the daily, weekly and monthly activities.

5

#### **5.2.2.5 Cell Maintenance**

The cells may adsorb analyte or impurities in the mobile phase over time. This process will slowly reduce the efficiency of the cells and can be minimized by ensuring that:

- The sample is filtered before injection.
- The mobile phase is filtered before use with a 0.2 micron filter membrane.
- The stationary phase is stable with regard to the mobile phase.
- The overall system is kept clean.

Removal of extraneous materials can frequently be performed by flushing the system with a solvent such as methanol or acetonitrile (the selection of the solvent will depend on the nature of the sample).

### **5.3 REPLACING SYSTEM COMPONENTS**

#### **5.3.1 Filter Elements**

When a flow cell is employed, filter elements may become clogged and must be replaced on a periodic basis. The frequency of replacement is dependent on the level of particulate matter present in the mobile phase and the sample, as well as the production of fine particles from the column.

If the filter must be replaced very frequently (e.g. more than once a week) it may be worthwhile to modify the composition of the mobile phase and/or switch to a more stable column (e.g. a column from a different manufacturer) which might create fewer fines.

Microbial growth may occur in mobile phases with low levels (<3%) of organic solvents unless suitable precautions are taken.

A daily log of system pressure should be kept so that any pressure fluctuations can be monitored, as this is a good indication of clogged filter elements.

To change a filter:

- a) Turn off the mobile phase flow and allow system pressure to drop to zero before disconnecting any components.



---

Caution: Do not remove the system pressure by opening a fitting on the high-pressure side of the column. The rapid pressure drop can damage various components in the overall system.

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- b) Remove the filter assembly from the chromatographic system by removing the nuts on either end of the assembly.
- c) Remove both end nuts from the filter assembly.
- d) Remove the used filter. If necessary, CAREFULLY insert a small wooden dowel or plastic rod to dislodge the filter.
- e) Rinse the filter housing with deionized water.
- f) Replace one end nut. Insert a new filter element into the filter housing. Ensure that the element is properly centered and seated against the surface of the end nut.
- g) Re-install the filter housing in the chromatographic system. Ensure that the direction of flow is as indicated on the filter housing.



---

Note: Initially, only the upstream end of the filter should be attached to the HPLC system. Pump about 5 mL of the mobile phase through the filter to waste before attaching the downstream end of the filter to the cell (this step will serve to wash the filter and ensure that particulate matter does not enter the cell).

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### 5.3.2 Changing the Line Fuse

If the unit does not power up when the main power switch is turned on or if the display is suddenly no longer illuminated, it is possible that a fuse has blown.



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Warning: Disconnect the system from the line power before removing the cover from the power input module. For continued protection against the risk of fire, replace only with the same type and rating of fuse.

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To replace a fuse:

- a) Remove the cover from the power input module using a small screwdriver or similar tool.
- b) Replace the fuse(s).
- c) Return the cover to the power module.

### 5.3.3 Replacing or Moving a Static Detector Module

To replace or move a static detector module:

- a) Turn off power to the system.
- b) Disconnect the cable connecting the detector module from the system board.
- c) Remove the two screws that attach the module from the base plate.

### 5.3.4 Replacing or Moving the Dynamic Detector

To replace or move a dynamic detector module:

- a) Turn off the power to the system.
- b) Disconnect the fiber optic cable connecting the detector module from the PD2000/DLS Module.
- c) Remove the two screws that attach the module from the base plate.

## 5.4 ESTABLISHING A SYSTEM LOG

A log that includes the usage and maintenance as well as any comments about operation of the system should be maintained. This log should include the date, time, technician's name, number of samples, any maintenance activities and any relevant user comments about the performance of the system.

A typical sample log is presented as Figure 5-1. If a prescribed sample log format is provided by your organization, that format could also be used to capture the relevant information.

Date	Time	User Name	Number Samples	Sample Type	Maintenance Activities	General Comments
7-3-02	11:22 AM	Jones	19	Unknowns	Daily Activities	OK
7-7-02	1:55 PM	Davis	27	BSA Standards 2319	Weekly activities	Leaky fitting repaired
7-7-02	11:33 PM	Davis	29	Compd 2301	Daily activities	OK
7-8-02	2:12 PM	Jones	21	Compd 2318	Daily activities	Significant peak tailing replaced column
7-9-02	4:28 PM	Wold	23	Compd 2400	Daily activities	OK

Figure 5-1: A Typical Log

## 5.5 TROUBLESHOOTING

### 5.5.1 Introduction to Troubleshooting

**Troubleshooting** refers to the determination of the cause of an abnormal condition or abnormal results from the system. The analyst should recognize that if a problem is observed, it might be due to the control module, the cells, the column, the solvent delivery system or to some other component of the system if an HPLC system is used to deliver the sample.

When the system is initially installed, the analyst should obtain a chromatogram of a well-defined sample before developing a new analytical procedure. This chromatogram can serve as a benchmark to be used to check the performance of the system. Similarly, if problems are observed in the use of a specific analytical procedure, it may be useful to use the standard sample to ensure that the chromatographic system is functioning properly.

### 5.5.2 Troubleshooting Guidelines

If the PDExpert system is connected to an HPLC system, it is important to understand that the system consists of several components and troubleshooting can be simplified by consideration of the following guidelines:

- a) In almost all cases, there is one proximate cause for the problem. As an example, if an increase in the baseline noise is observed, the problem can be caused by one of the following:
  - The pump (e.g. the pump is not primed)
  - The mobile phase (the mobile phase is not suitably degassed)
  - The column (the column is contaminated and strongly eluted compounds are being eluted)
  - The detector (there is an electronic problem)
  - The cell may be contaminated
  - A fitting (a fitting may be leaking)
- b) A fundamental knowledge of the role of each component of the system is extremely useful in diagnosing the problem.
- c) The availability of spare parts to substitute is very useful in diagnosing the problem.
- d) If a problem is observed, run a "standard" sample to determine if the problem is instrument related or analysis related.
- e) If any aspects of the analytical conditions is to be changed, run a "before" and "after" to ensure that the effect of the change is well understood. Do not consider any change as "trivial". As an example, if you change the supplier of a buffer salt, verify that the change has no effect on the analysis.
- f) To isolate the source of the problem, it may be valuable to perform independent checks of each of the components in the HPLC system.

A series of diagnostic procedures is presented below that will assist in pinpointing the cause of the problem. Since some problems from the pump or column are observed via the detector, we include a detailed discussion of potential problems for a typical system which includes HPLC with light scattering detection.

### 5.5.3 Erratic/Noisy Baseline

Cause	Comments	Solution
Dissolved gases in the pumphead	If dissolved gases come out of solution in the pumphead the flow rate will be variable. This will cause cyclic noise. The frequency of the pattern will increase as the flow rate is increased.	Sparge the mobile phase with a brisk flow of He for a few minutes or degas it via an ultrasonic bath.
Dissolved gases in the detector cell	If dissolved gases come out of solution in the detector cell, sharp noise spikes may be observed.	Increase the flow rate for 30 min to remove gases.  Use a vacuum degasser.  Remove the detector from the system, flush it with water and then with degassed MeOH and again with water.
Pump Head Problem	Check pump seals/check valves for wear or leaks.	Replace seals if worn.  Replace check valves if necessary.
Leaks in the system	Check for leaks in system.	Tighten all fittings.
Mobile phase not properly mixed		Stir mobile phase.
Contaminants eluting from column	Trace levels of organic compounds may be tightly retained by the column.	Remove column and see if problem exists. Clean the column and/or replace.
System not grounded	All components of the system must be connected to a common ground.	Check AC line receptacle - verify that you have a true ground.
Cell Temperature not held constant	Temperature variation, potentially due to a temperature change in laboratory	Maintain cells at constant temperature.
Detector channel is misaligned	If one channel is noisy or erratic	Realign detector module (contact service)
Laser is misaligned	If all channels are noisy or erratic	Realign laser (contact service)

#### 5.5.4 High Background Signals

This section describes situations where the background signal has noticeably increased in a short period of time.

Cause	Comments	Solution
Impurities in the mobile phase		Select a new supply of mobile phase
Leaking Cell		Check for wetness around cell and tighten fittings or replace cell.
Contaminants leaching from system components		Check mobile phase reservoir filters, column end frits, replace if necessary.
Detector channel is misaligned	If one channel is noisy or erratic	Realign detector module (contact service)
Laser is misaligned	If all channels are noisy or erratic	Realign laser (contact service)



### 5.5.5 Increase in Back Pressure

Cause	Comments	Solution
Accumulation of particulates from the mobile phase or injected samples		Replace in-line filter elements.  Ensure that the mobile phase and/or samples are filtered through a 0.22 µm Nylon or PVDF membrane filter. Use a mobile phase with a substantial fraction of an organic solvent to prevent bacterial growth.  Use freshly prepared mobile phase. Bacterial growth in the mobile phase may lead to clogging of the filter.
Clogged injector or column		Isolate suspect component.  Refer to manufacturer's cleaning directions or replace rotor seal and/or stator face on injector.  Ensure that the mobile phase and sample are filtered before use.
Plugged Tubing		Isolate plugged tubing and replace.
Clogged cell		Remove cell from system and check back pressure.  Clean cell.

### 5.5.6 Loss of Response

This section describes the abrupt loss of a peak or peaks from the chromatogram when using a set of analytical conditions which is known to provide a useful chromatogram.

Cause	Comments	Solution
Accidental change of a parameter in the software		Check settings.
Compounds of interest not sufficiently stable	Some compounds will decompose as a function of time	Check stability as a function of time, and prepare fresh standards. If necessary, change conditions.  Use a cooled autosampler.
Change in pH or mobile phase composition	Mobile phases should be freshly prepared	Check mobile phase and prepare fresh phases.
The injector may be partially or fully clogged	Make sure that the sample and mobile phase are clean	Clean the injector.
Detector channel is misaligned	If one channel is noisy or erratic	Realign detector module (contact service)
Laser is misaligned	If all channels are noisy or erratic	Realign laser (contact service)

### 5.5.7 Inability to Autozero the Signal

Cause	Comments	Solution
Autozero has been performed on a very noisy signal		Reduce the noise or increase the current gain range.
Detector channel is misaligned	If one channel is noisy or erratic	Realign detector module (contact service)
Laser is misaligned	If all channels are noisy or erratic	Realign laser (contact service)

## Appendix A

### Specifications

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Sample Cell	35 $\mu$ L flow through or 6 mm test tube (others available)
Light Scattering Focused Volume	20 x 60 microns
Sequential Static Angle Measurements	up to 24 angles available
Available Measurement Angles	Both sides of the plain 5 degree increments excluding 0 and 180 degrees
DLS - Hydrodynamic Radius Option	0.5 to 1000 nm
Temperature Range	0 to 80 degrees C Standard
Temperature Stability	$\pm$ 0.10 degrees C
Laser Life	9,000 hours in normal operation
SPCM Count Rate	6 MHz (maximum)
Platform Footprint	28 inches x 31 inches, 71 cm x 79 cm
Stand-alone Weight (a)	45 kg (100 lb)
Power Requirement	5 VA, 120-240 V, 50-60 Hz

(a) Approximate weight, depending on configuration

**A**

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## Appendix B

### Spare Parts and Replacement Parts

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**B**

#### Consumables

Part Number	Description
KIT-PDE-5001	6 mm Test Tube (pack of 10)
KIT-PDE-5002	6 mm Test Tube (pack of 50)
	Index Matching Fluid

#### Replacement Parts

Part Number	Description
ASY-PDE-2021	Cell Ring Assembly (Cuvette)
AST-PDE-2022	Cell Ring Assembly (Flow Cell)

#### Accessories

Part Number	Description
ASY-PDE-3030	Static Detector Assembly
AST-PDE-3032	DLS Detector Assembly

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## Appendix C

### General Principles of Dynamic Light Scattering

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#### C.1 WHAT IS LIGHT SCATTERING?

C

The propagation of light may be considered as a continuous rescattering of the incident electromagnetic wave from every point of the illuminated medium. The amplitude of each secondary wave is proportional to the polarizability at the point from which this wave originates; if the medium is uniform, rescattered waves will have the same amplitude and interfere destructively in all directions except in the direction of the incident beam. If, however, at some location the index of refraction differs from the average value, the wave that is rescattered at this location is not compensated for and some light will be observed in directions other than the direction of incidence and light scattering occurs. Scattering of light can be viewed as a result of microscopic heterogeneities within the illuminated volume; and macromolecules and supramolecular assemblies are examples of such heterogeneities.

#### C.2 LIGHT SCATTERING TECHNIQUES

Static light scattering probes concentration, molecular weight, size, shape, orientation, and interactions among scattering particles by measuring the average intensity and polarization of the scattered light. Static light scattering measurements which are performed at different scattering angles provide information on the molecular weight, size, and shape of the scattering particles. Measurements of the intensity of light scattering as a function of concentration yield the second virial coefficient, which is the key characteristic of the strength of attractive or repulsive interactions between solute particles.

Quasielastic (dynamic) light scattering<sup>1,2</sup> probes the relatively slow fluctuations in concentration, shape, orientation and other particle characteristics by measuring the correlation function of the scattered light intensity. Fast vibrations of small chemical groups which lead to significant changes in the frequency of the scattered light is the domain of Raman spectroscopy. These latter two methods, which probe the dynamics of the particles which cause light scattering, are intrinsically more complicated than static light scattering, since they involve measurements of spectral characteristics or related correlation properties of the scattered light.

### C.3 LIGHT SCATTERING FROM MACROMOLECULES IN SOLUTION

One may consider the solution as a homogeneous medium and ascribe light scattering to the spatial fluctuations in the concentration of a solute. An alternative way is to consider each individual solute particle as a heterogeneity and therefore as a source of light scattering. The first approach is more appropriate for solutions of small molecules in which the average distance between the center of the scatterers is small compared to the wavelength of light. The second approach is more appropriate for solutions of large macromolecules and colloids, when the average distance between particle centers is comparable to the wavelength of light. When the size of the solute particles becomes comparable to the wavelength of light, the description of the effects of orientational motion and deformation of the solute particles is much more straightforward when these particles are treated as individual scatterers.

Intensity of the light scattered by a single particle is dependent on the mass and the shape of the particle. In this discussion, we will consider an aggregate composed of  $N$  monomers and the amplitude of the electromagnetic wave scattered by an individual monomer is  $A_0$  (at the point of observation). If the size of the aggregate is small compared to the wavelength of light ( $\lambda$ ), all waves scattered by individual monomers interfere constructively and the resulting wave has an amplitude  $NA_0$ . Since the intensity of a light wave is proportional to its amplitude squared, the intensity of the light scattered by the aggregate is proportional to the aggregation number squared,  $I = N^2 I_0$ , where  $I_0$  is the intensity of scattering by a monomer. The quadratic dependency of scattering intensity on the mass of the scatterer is the basis for optical determination of the molecular weight of macromolecules. It is this dependency which is accounted for by the Mass Normalization function of *PrecisionDeconvolve*.

If the size of an aggregate particle is not small compared to  $\lambda$ , the interference of the electromagnetic waves scattered by the constituent monomers is not all constructive and the phases of these waves must be taken into account. If the phase of a wave scattered at the origin is used as a reference, the phase of a wave scattered at a point with radius vector  $\mathbf{r}$  is  $\mathbf{k} \cdot \mathbf{r}$  as shown in Figure C-1). The vector  $\mathbf{k}$  is called the “scattering vector“, which is a fundamental characteristic of any scattering process. The length of the vector is indicated in equation C-1.

#### C-1

where:  $n$  is the refractive index of the medium  
 $\lambda$  is the wavelength of light  
 $\theta$  is the scattering angle

Partial cancellation of waves scattered by different parts of the large aggregate reduces the intensity of light scattering by a factor of  $S$ , where  $S$  is an averaged value of the phase factors  $e^{i\mathbf{k} \cdot \mathbf{r}}$  for all monomers. The factor  $S$  should be averaged over all possible orientations of the particle. The result of this averaging yields the structure factor,  $S$ . Expressions for the structure factors for particles of various shapes can be found elsewhere.<sup>3</sup>

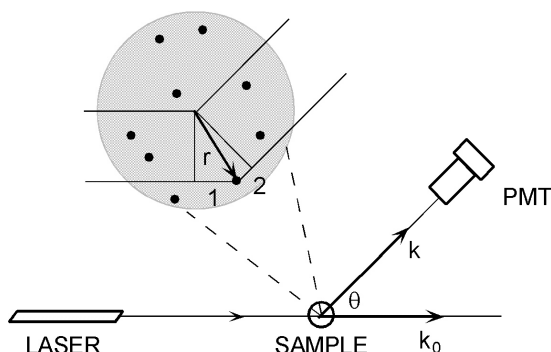




Figure C-1: The Scattering Vector

The path traveled by a wave scattered at the point with radius vector  $\mathbf{r}$  differs from the path passing through the reference point O by two segments, 1 and 2, with lengths  $r_1$  and  $r_2$ , respectively. The phase difference is  $\Delta\phi$  where  $k$  is the absolute value of the wave vector  $\mathbf{k}$  (or  $2\pi/\lambda$ ). The segment  $r_2$  is a projection of  $\mathbf{r}$  on the wave vector of the incident beam  $\mathbf{k}_i$ , i.e.  $r_2 = \mathbf{r} \cdot \mathbf{k}_i / k_i$ . Similarly,  $r_1 = \mathbf{r} \cdot \mathbf{k}_s / k_s$ , and thus  $\Delta\phi = \mathbf{k} \cdot \mathbf{r}$ . Vector  $\mathbf{k}$  is called the scattering vector.

## C.4 METHOD OF QUASIELASTIC LIGHT SCATTERING SPECTROSCOPY (QLS)

### C.4.1 The Motion of Particles in Solution

C

When light is scattered from a collection of  $N$  solute molecules, at the observation point we also have a sum of waves scattered by individual particles (Figure A-1). Each particle could be at any random location within the scattering volume (the intersection of the illuminated volume and the volume from which the scattered light is collected). Since the size of the scattering volume is much bigger than  $\lambda^{-1}$  (with the exception of nearly forward scattering, where  $\theta \approx 0$ ), the phases of the waves scattered by different particles will vary dramatically. As a result, the average amplitude of the scattered wave is proportional to  $N^{1/2}$  and the average intensity of the scattered light is simply  $N$  times the intensity scattered by an individual particle, as expected. The local intensity, however, fluctuates from one point to another around its average value. The spatial pattern of these fluctuations in light intensity, called an interference pattern or “speckles”, is determined by the positions of the scattering particles. As the scattering particles move, the interference pattern changes in time resulting in temporal fluctuations in the intensity of light detected at the observation point. The essence of the QLS technique is to measure the temporal correlations in the fluctuations in the scattered light intensity and to reconstruct from these data the physical characteristics of the scatterers.

### C.4.2 Coherence Area

There is a characteristic size for speckles in the interference pattern. If the intensity of the scattered light is above average at a certain point it will also be above the average within an area around this point where phases of the scattered waves do not change significantly; this area is called the coherence area. Within different coherence areas, the fluctuations in intensity of light collected are statistically independent. Increasing the size of the light-collecting aperture beyond the size of a coherence area does not lead to improvement of the signal-to-noise ratio because the temporal fluctuations in the intensity are averaged out. For a monochromatic source, the scattered light is coherent within a solid angle of the order of  $\sqrt{\lambda^2/A}$ , where  $A$  is the cross-sectional area of the scattered volume perpendicular to the direction of the scattering. Because the coherence angle is fairly small, powerful (100 mW) and well-focused laser illumination, and photon counting techniques, are used in the PDI/BATCH instrument.

### C.4.3 The Correlation Function

While the photodetector signal in QLS is random noise, information is contained in the correlation function of this random signal. The correlation function of the signal  $I(t)$ , which in the particular case of QLS is the photocurrent, is defined in equation C-2.

$$\text{C-2}$$

The notation  $\langle \dots \rangle$  is introduced to distinguish the correlation function of the photocurrent from the correlation function of the electromagnetic field  $E(t)$  (which is the Fourier transform of the light spectrum):

$$\text{C-3}$$

In the above formulae, the angular brackets denote an average over time  $\tau$ . This time averaging, an inherent feature of the QLS method, is necessary to extract information from the random fluctuations in the intensity of the scattered light.

For very large delay times  $\tau$ , the photocurrents at moment  $t$  and  $t + \tau$  are completely uncorrelated and  $\langle I(t)I(t + \tau) \rangle$  is simply the square of the mean current  $\bar{I}^2$ . At  $\tau = 0$ ,  $\langle I(t)I(t) \rangle$  is obviously the mean of the current squared  $\overline{I^2}$ . Since for any  $\tau$ , the initial value of the correlation function is always larger than the value at a sufficiently long delay time. The characteristic time within which the correlation function approaches its final value is called correlation time. For example, in the most practically important case of a correlation function that decays according to an exponential law  $\exp(-\tau/\tau_c)$ , the correlation time is the parameter  $\tau_c$ .

In the majority of practical applications of QLS, the scattered light is a sum of waves scattered by many independent particles and therefore displays Gaussian statistics. This being the case, there is a relation between the intensity correlation function  $\langle I(t)I(t + \tau) \rangle$  and the field correlation function  $\langle E(t)E(t + \tau) \rangle$ :

$$\text{C-4}$$

Here  $\bar{I}$  is the normalized field correlation function,  $\bar{I}$  is the average intensity of the detected light, and  $\eta$  is the efficiency factor. For perfectly coherent incident light and for scattered light collected within one coherence area, the efficiency factor is 1. If light is collected from an area  $J$  times larger than the coherence area, fluctuations in light intensity are averaged out and the efficiency factor is of the order of  $1/J \ll 1$ . Low efficiency makes the quality of measurements vulnerable to fluctuations in the average intensity caused by the presence of large dust particles in the sample or instability of the laser intensity.

#### C.4.4 Determination of the Correlation Function

In PDI instruments the correlation function is determined digitally. The number of photons registered by the photodetector within each of a number of short consecutive intervals is stored in the correlator memory. Each count in a given interval (termed the "sample time" and denoted  $\Delta t$ ) represents the instantaneous value of the photocurrent  $i(t)$ . The series of  $i(t)$  counts held in the correlator memory is termed the "digitized copy" of the signal. According to Equation (1), to obtain the correlation function  $g(\tau)$  at  $\tau$ , the average product of counts separated by  $\tau$  sample times should be determined. The number  $n$  is referred to as a channel number. Up to  $n$  channels, in principle, can be measured simultaneously, but usually a smaller subset of  $n$  equidistant or logarithmically-spaced channels is used. Clearly, the shortest delay time at which the correlation function is measured by the procedure described above is  $\Delta t$  (channel 1). The longest delay time cannot exceed the duration of the digitized copy,  $T$ . Thus, it is important that the correlation time  $\tau$  fit into the interval  $T$ . This condition determines the choice of the sample time for the particular measurement.

To increase the statistical accuracy with which the correlation function is determined, it is essential to maximize the number of count pairs whose products are averaged within the measurement time. If the correlation function is being measured in  $n$  channels simultaneously, ideally  $n$  products should be processed for each new count, i.e. during sample time  $\Delta t$ . The instrument capable of doing this is said to be working in the "real time regime". The real time regime means that the information contained in the signal is processed without loss. The PDI correlator works in real time with a minimal sample time  $\Delta t$  of 1 microsecond and the length of the digital copy  $T = 1024 \Delta t$ . The number of channels  $n$  processed in real time is determined by formula (2) and cannot exceed 256.

#### C.4.5 Brownian Motion

Temporal fluctuations in the intensity of the scattered light are caused by the Brownian motion of the scattering particles. The speed of the particles is related to the size, small particles move faster than large particles. Though each particle moves randomly; in a unit time more particles leave regions of high concentration than leave regions of low concentration. This results in a net flux of particles along the concentration gradient. Brownian motion is thus responsible for the diffusion of the solute and is quantitatively characterized by the diffusion coefficient,  $D$ . The laws of diffusive motion stipulate that over time  $t$  the displacement  $x$  of a Brownian particle in a given direction is characterized by the relationship  $x = \sqrt{2Dt}$ .

#### C.4.6 Determination of the Diffusion Coefficient D

As explained earlier, temporal fluctuations in scattered light intensity are caused by the relative motions of particles in solution. Two spherical waves scattered by a pair of individual particles have, at the observation point, a phase difference of  $\Delta\phi$ , where  $r$  is the (vector) distance between particles. As the scattering particles move over distance  $\Delta r$  along the vector  $\mathbf{q}$ , the phases for all pairs of particles change significantly and the intensity of the scattered light becomes completely independent of its initial value. The correlation time,  $\tau_c$ , is thus the time required for a Brownian particle to move a distance  $\Delta r$  along the vector  $\mathbf{q}$ . As stated above,  $\Delta r = \sqrt{2Dt}$ , thus for  $\tau_c$ ,  $\Delta r = \sqrt{2D\tau_c}$ . Rigorous mathematical analysis of the process of light scattering by Brownian particles leads to the following expression for the correlation function of the scattered light:

$$C-5$$

#### C.4.7 Determination of the Sizes of Particles in Solution

According to Equations C-4 and C-5, measurement of the intensity correlation function allows evaluation of the diffusion coefficients of the scattering particles. The diffusion coefficient in an infinitely dilute solution is determined by particle geometry. For spherical particles, the relation between the radius  $a$  and its diffusion coefficient  $D$  is given by the Stokes-Einstein equation:

$$C-6$$

where:  $k_B$  is the Boltzmann constant  
 $T$  is the absolute temperature  
 $\eta$  is the viscosity of the solution

For non-spherical particles it is customary to introduce the apparent hydrodynamic radius  $a_h$ , defined as:

$$C-7$$

where:  $D$  is the diffusion coefficient measured in the QLS experiment.

For non-spherical particles, it is important to note that the diffusion coefficient is actually a tensor—the rate of particle diffusion in a certain direction depends on the particle orientation relative to this direction. As small particles, diffuse over a distance  $\Delta r$ , their orientation may be changed many times. QLS measures the average diffusion coefficient for these particles. Particles of a size comparable to, or larger than,  $\Delta r$  essentially preserve their orientation as they travel a distance smaller than their size. For these particles, the single exponential expression of equation A-5 for the field correlation function is not strictly applicable.

For particles that are small compared to  $\Delta r$ , the hydrodynamic radius is calculated numerically, and in some cases analytically, for a variety of particles shapes. The important analytical formula for the prolate ellipsoid, with the long axis  $a$  and the ratio of lengths of the short axis to the long axis  $p$  is :

$$C-8$$

The above formulae connecting the diffusion coefficient or hydrodynamic radius to particle geometry are strictly applicable only for infinitely dilute solutions. At finite concentrations, two additional factors significantly affect the diffusion of particles: viscosity and interparticle interactions. Viscosity generally increases with the concentration of macromolecular solute. According to equation A-6, this leads to a lower diffusion coefficient and therefore to an increase in the apparent hydrodynamic radius. Interactions

between particles can act in either direction. If the effective interaction is repulsive, which is usually the case for soluble molecules (otherwise they would not be soluble), local fluctuations in concentration tend to dissipate faster, meaning higher apparent diffusion coefficients and lower apparent hydrodynamic radii. If the interaction is attractive, fluctuations in concentration dissipate slower and the apparent diffusion coefficients are lower. Thus, depending on whether the effect of repulsion between particles is strong enough to overcome the effect of increased viscosity, both increasing and decreasing types of concentration dependence of the hydrodynamic radius are observed.<sup>4</sup> In this context, it should be noted that the interaction between large particles (as compared to ) generally leads to a non-exponential correlation function that does not take the form of equation C-4 and therefore cannot be completely described by a single parameter .

## **C.5 DATA ANALYSIS**

### **C.5.1 Polydispersity and the Mathematical Analysis of QLS Data**

Polydispersity can be an inherent property of the sample, for instance when polymer solutions or protein aggregation are studied, or it can be a consequence of impurities or deterioration of the sample. In the first case, the polydispersity itself is often an object of interest, while in the second case it is an obstacle. In both instances, polydispersity significantly complicates data analysis.

For polydisperse solutions, equation A-5 for the normalized field correlation function must be replaced with:

$$\text{C-9}$$

In this expression,  $D_i$  is the diffusion coefficient of particles of the  $i$ -th kind and  $I_i$  is the intensity of light scattered by all of these particles.  $N_i$ , where  $N$  is the number of particles of  $i$ -th kind in the scattering volume and  $I_i$  is the intensity of the light scattered by each such particle. For a continuous distribution of scattering particle size, equation C-10 is generalized as follows:

where:  $I$  is the intensity of light scattered by particles having their diffusion coefficient in the interval  $[D, D+dD]$   
 $N(D)dD$  is the number of these particles in the scattering volume  
 $I_0$  is the intensity of light scattered by each of them.

The goal of the mathematical analysis of QLS data is to reconstruct as precisely as possible the distribution function  $N(D)$  (or  $I(D)$ ) from the experimentally measured function  $I(q, \omega)$ .

It should be noted that polydispersity is not the only source of non-single exponential correlation functions of scattered light. Even in perfectly monodisperse solutions, interparticle interactions, orientation dynamics of asymmetric particles, and conformational dynamics or deformations of flexible particles will lead to a much more complicated correlation function than described by equation A-6. These effects are usually insignificant for scattering by particles small compared to the length of the inverse scattering vector  $q$ , but become important, and often overwhelming, for larger particles. In those cases, QLS probes not the pure diffusive Brownian motion of the scatterers, but also other types of dynamic fluctuation in the solution.

### C.5.2 Deconvolution of the Correlation Function, an “Ill-Posed” Problem

The values of  $I(q, \omega)$  contain statistical errors. We have described previously the features of the QLS instrument that are essential for minimizing these errors. It is equally important to minimize the distorting effect that experimental errors in  $I(q, \omega)$  have on the reconstructed distribution function  $N(D)$ . The distribution  $N(D)$  is a non-negative function. *A priori* then, a non-negative function  $N(D)$  should be sought that produces, via Equations A-3 and A-10, the function  $I(q, \omega)$  which is the best fit to the experimental data. Unfortunately, this simplistic approach does not work. The underlying reason is that the corresponding mathematical minimization problem is “ill-posed,”<sup>5</sup> meaning that dramatically different distributions lead to nearly identical correlation functions of the scattered light and therefore are equally acceptable fits to the experimental data. For example, addition of a fast oscillating component to the distribution function  $N(D)$  does not change  $I(q, \omega)$  considerably since the contributions from closely spaced positive and negative spikes in the particle distribution cancel each other. We discuss below three approaches for dealing with this ill-posed problem.

### C.5.3 The Direct Fit Method

The simplest approach is the direct fit method. In this method, the functional form of  $N(D)$  is assumed *a priori* (single modal, bimodal, Gaussian, etc.) and the parameters of the assumed function that lead the best fit of  $I(q, \omega)$  to  $I(q, \omega)$  then are determined. This method is only as good as the original guess of the functional form of  $N(D)$ . Moreover, using the method can be misleading because it may confirm nearly any *a priori* assumption made. It is also important to note that the more parameters there are in the assumed functional form of  $N(D)$ , the better the experimental data can be fit but the less meaningful the values of the fitting parameters become. In practice, typical QLS data allow reliable determination of about three independent parameters of the size distribution of the scattering particles.

### C.5.4 The Method of Cumulants

The second approach is not to attempt to reconstruct the shape of the scattering particle distribution but instead to focus on so-called “stable” characteristics of the distribution, i.e. characteristics which are insensitive to possible fast oscillations. In particular, these stable characteristics are moments of the

distribution, or closely related quantities called cumulants.<sup>12</sup> The first cumulant (moment) of the distribution, that gives the average diffusion coefficient, can be determined from the initial slope of the field correlation function. Indeed, using equation C-12, it is straightforward to show that:

### C-12

The second cumulant (moment) of the distribution can be obtained from the curvature (second derivative) of the initial part of the correlation function. As in the direct fit method, the accuracy of the real QLS experiment allows determination of at most three moments of the distribution. The first moment, can be determined with better than  $\pm 1\%$  accuracy. The second moment, the width of the distribution, can be determined with an accuracy of  $\pm 5\text{-}10\%$ . The third moment, which characterizes the asymmetry of the distribution, usually can be estimated with an accuracy of only about  $\pm 100\%$ .

**C**

### C.5.5 Regularization

The regularization approach combines the best features of both of the previous methods. The advantage of the cumulant method is that it is completely free from bias introduced by *a priori* assumptions about the shape of, assumptions that are at the heart of the direct fit method. On the other hand, reliable *a priori* information on the shape of the distribution function, in addition to the experimental data, improves significantly the quality of results obtained by the QLS method. The regularization method assumes that the distribution is a smooth function and seeks a non-negative distribution producing the best fit to the experimental data. As discussed above, the ill-posed nature of the deconvolution problem means that distributions differing by the presence or absence of a fast oscillating function produce very similar correlation functions. The regularization requirement that the distribution should be sufficiently smooth eliminates this ambiguity, allowing unique solutions to the minimization problem. There are several methods that utilize this approach for reconstructing the scattering particle distribution function from QLS data. All of these methods impose the condition of smoothness on the distribution but differ in the specific mathematical approaches used for this purpose. One popular program, originally developed by Provencher, is called CONTIN.<sup>6</sup> Precision Detectors use a proprietary algorithm of superior quality.

All regularization algorithms produce similar results and incorporate the use of a parameter that determines how smooth the distribution has to be. The choice of this parameter is one of the most difficult and important parts of the regularization method. If the smoothing is too strong, the distribution will be very stable but will lack details. If the smoothing is too weak, false spikes can appear in the distribution. The “rule of thumb” is that the smoothing parameter should be just sufficient to provide stable, reproducible results in repetitive measurements of the same correlation function. Two facts are helpful for choosing the appropriate smoothing parameter. First, the lower are the statistical errors of the measurements, the smaller the smoothing parameter can be without loss of stability. This will yield finer resolution in the reconstructed distribution. Second, narrow distributions generally require much less smoothing and can be reconstructed much better than can wide distributions. This is because oscillations in narrow distributions are effectively suppressed by non-negativity conditions.

The moments of the distribution reconstructed by the regularization procedure coincide closely with those obtained by other methods. However, the regularization procedure, in addition, gives unbiased (apart from smoothing) information on the shape of the distribution. This shape cannot be extracted through use of the direct fit method, nor from cumulant analysis. In a typical QLS experiment, regularization analysis can resolve a bimodal distribution with two narrow peaks of equal intensity if the diffusion coefficients corresponding to these peaks differ by more than a factor of  $\sim 2.5$ .

## FOOTNOTES

<sup>1</sup> R. Pecora, "Dynamic Light Scattering: Applications of Photon Correlation Spectroscopy." Plenum Press, New York, 1985.

<sup>2</sup> K. S. Schmitz, "An Introduction to Dynamic Light Scattering by Macromolecules." Academic Press, Boston, 1990.

<sup>3</sup> H. C. van de Hulst, "Light Scattering by Small Particles." Dover, New York, 1981.

<sup>4</sup> A. N. Tikhonov and V. Y. Arsenin, "Solution of Ill-Posed Problems." Halsted Press, Washington, 1977.

<sup>5</sup> D. E. Koppel, *J. Chem. Phys.* **57**, 4814 (1972).

<sup>6</sup> S. W. Provencher, *Comput. Phys. Commun.* **27**, 213 (1982).



## Appendix D

### Multi-Angle Light Scattering to Determine $M_w$ and $\langle r^2 \rangle$ – The Zimm Plot

---

The discussions about static light scattering and dynamic light scattering that are presented in Chapters 5 and 6 are based on a number of assumptions including:

**D**

- the particles are monodisperse.
- the particles are present at infinite dilution.
- the solvent does not affect the configuration of the polymer.

The analyst will note that in many cases there is at least some degree of polydispersity of the polymer and it is known that the configuration of many polymers is affected by the nature of the solvent. In addition, the particles are not present at infinite dilution and the light scattering phenomena may be affected re may be affected by interparticle interaction and/or the interference between the light scattered by different particles.

While the assumptions are useful as they simplify data analysis, more advanced approaches may be more valid. Collecting light scattering data at several angles and using the Zimm plot can lead to the determination of more accurate physical properties.

The scattering of light by a solution of polymers as a function of the size of the random coil is presented as equation D-1.

$$\frac{Kc}{R_\theta} = \frac{1}{M_w} + \frac{1}{M_w} \frac{16}{3} \pi^2 \frac{\langle r^2 \rangle}{6\lambda^2} \sin^2 \frac{\theta}{2} + 2A_2 c \quad \text{D-1}$$

where: **n** is the refractive index of the solvent  
**c** is the concentration of polymer  
**N<sub>A</sub>** Avagadro's number  
 $\lambda_o$  is the wavelength of light (in vacuo)  
 $\lambda$  is the wavelength of light (in the medium)  
 $\theta$  is the scattering angle  
**f** is a correction factor  
**A<sub>2</sub>** is a constant

$R_\theta$  is the reduced scattered intensity, which is defined in equation D-2.

$$R_\theta = r^2 \frac{I_\theta}{I_0} \quad \text{D-2}$$

where:  $r$  is the distance between the sample and the point where the intensity  $I_\theta$  is recorded and  $I_0$  is the intensity of the incoming light.

This equation allows for separate determination of  $M_w$  and  $\langle r^2 \rangle$  via equations D-3 and D-4.

$$\text{D-3}$$

$$\lim_{\theta \rightarrow 0} \frac{Kc}{R_\theta} = \frac{1}{M_w} + \frac{1}{M_w} \frac{16}{3} \pi^2 \frac{\langle r^2 \rangle}{6\lambda^2} \sin^2 \frac{\theta}{2} \quad \text{D-4}$$

From an operational perspective, data is collected at a number of angles for several concentrations and then plotted as shown in Figure D-1 (the Zimm plot).

The molecular weight and the radius is obtained by extrapolating the data to concentration = 0 and  $\Theta = 0$  and applying equations D-3 and D-4.

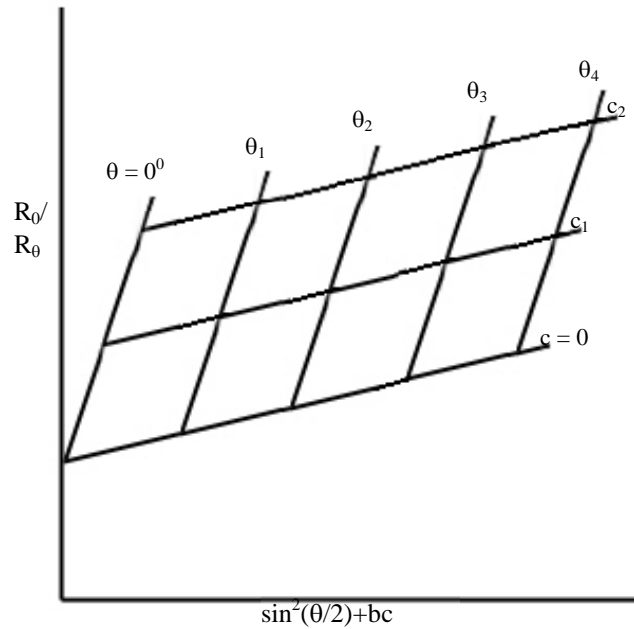


Figure D-1: A Schematic Zimm Plot

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